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PENSOFT

First record of *Vallonia costata* (O.F. Müller, 1774) (Gastropoda, Eupulmonata, Valloniidae) from British Columbia, Canada, confirmed by partial-COI gene sequence

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Abstract
The minute terrestrial snail *Vallonia costata* (O.F. Müller, 1774) is reported for the first time from the province of British Columbia, Canada. The identification was based on shell morphology and confirmed by COI gene data. This species is presumed to be introduced to the province.

Key words
Cytochrome *c* oxidase subunit I; introduced species; Pupilloidea; range extension; terrestrial snail.

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Introduction

The genus *Vallonia* Risso, 1826 (Gastropoda, Eupulmonata, Valloniidae) is a group of small, whitish terrestrial snails distributed in the Northern Hemisphere. The monograph by Gerber (1996) included 35 species and more have been described since (e.g. Meng and Gerber 2008, Gerber and Bössneck 2009). Shell morphology is traditionally used to distinguish species (e.g. Pilsbry 1948, Gerber 1996). However, Korte and Armbruster (2003) concluded after using molecular sequence data (ITS-1 rDNA) that at least 1 nominal species, *V. excentrica*, is paraphyletic, consisting of a group of genetically different forms with a similar conchological phenotype. Among these and other genera of small snails, such as *Carychium* O.F. Müller, 1773 (Ellobiidae) (Weigand et al. 2010), *Cochlicopa* A. Férussac, 1821 (Cochlicopidae) (Armbruster 1997), and *Pyramidula* Fitzinger, 1833 (Pyramidulidae) (Razkin et al. 2017), shell characters cannot always be relied on for accurate determinations of species. Although taxonomy of microsnails has until recently been largely conchologically driven, molecular methods and integrative taxonomy employing multiple lines of data have become standard in malacological works (Jochum et al. 2015, Dayrat et al. 2016). In the genus *Vallonia*, conchologically based species determinations still prevail. The latest work encompassing all *Vallonia* taxa, except for those named subsequently, is the detailed monograph by Gerber (1996). However, except for descriptions of shell morphology, some basic ecology, little is known about *Vallonia* species in North America (see Pilsbry 1948), and no molecular work on the genus on this continent has been published.

The distribution of *Vallonia costata* (O.F. Müller, 1774) includes much of Western Europe and a portion of eastern Canada and the United States (Pilsbry 1948,

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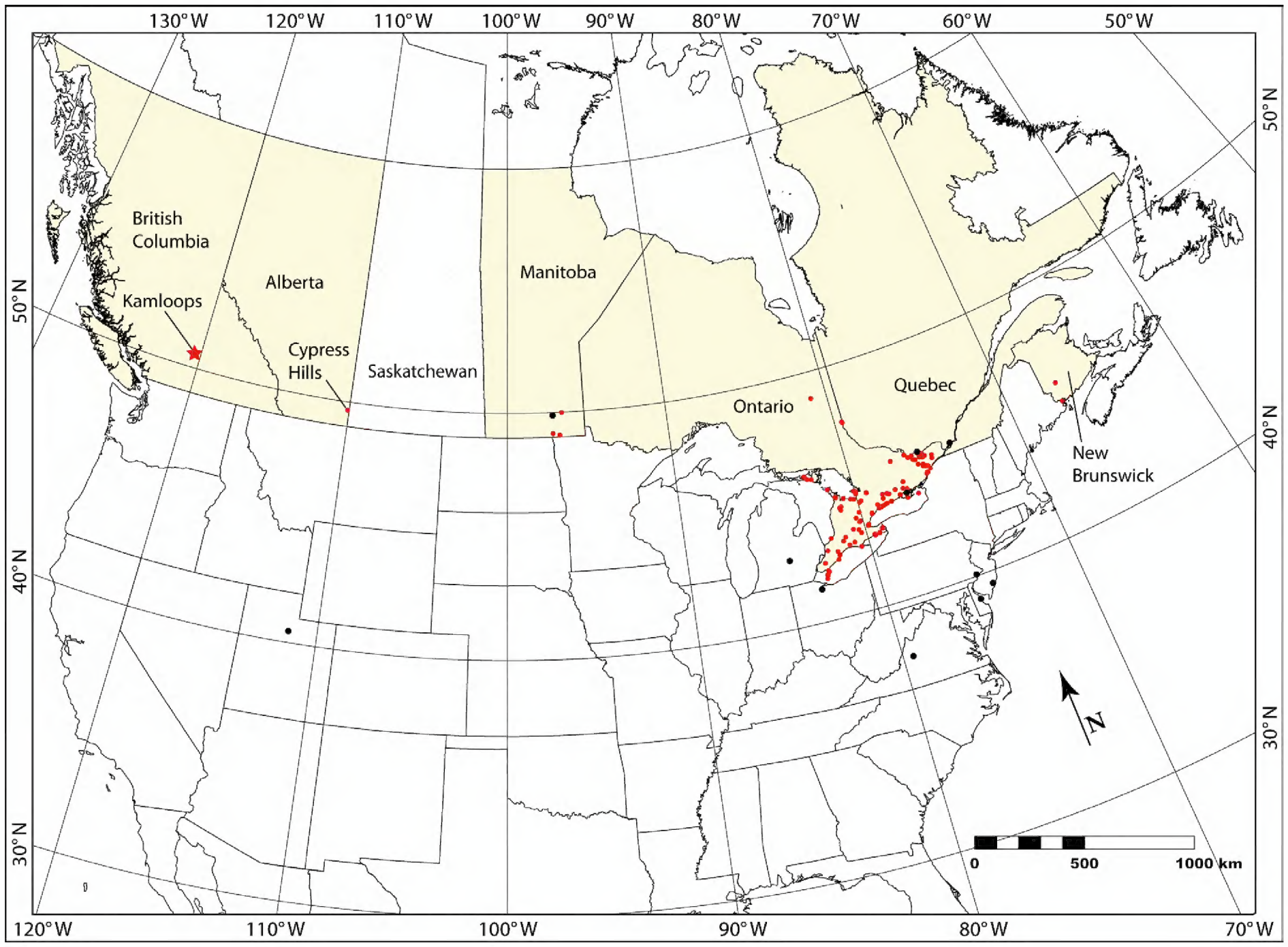

Figure 1. Canadian distribution of *Vallonia costata* based on Gerber's (1996) list of materials examined (black dots) and the Forsyth collection (red dots). The new records from British Columbia, Canada are represented by the red star).

Hubricht 1985, Gerber 1996). If we follow the general consensus (e.g. Pilsbry 1948, Hubricht 1985, Gerber 1996), the eastern North American occurrences of *V. costata* are native, not introductions. However, it is clear that *V. costata* is strongly synanthropic in these areas, which suggests that it may be an old introduction, as Nekola et al. (2015) proposed for *Pupilla muscorum* (Linnaeus, 1758) in North America. Gerber (1996) cited records from the provinces of Ontario, Quebec, and Manitoba, but his map is quite imprecise and seems to show the range extending westwards at least into Saskatchewan, probably based on literature records.

Eight species of *Vallonia* are known from Canada (Grimm et al. 2010). In British Columbia (BC), 4 species of *Vallonia* have been recognized using shell morphology: 2 unribbed species, *V. pulchella* (O. F. Müller, 1774) and *V. excentrica* (Sterki *in* Pilsbry, 1893), and 2 ribbed species, *V. cyclophorella* (Sterki, 1892) and *V. gracilicosta* Reinhardt, 1883 (Forsyth 2004). Individually, these 2 groups (ribbed and unribbed) both present difficulties for identification.

Here, we record *V. costata* from Kamloops, British Columbia, Canada, using molecular data and shell morphology to identify this species. This represents a first record of *V. costata* from the province of British Columbia (Fig. 1), where we expect it is introduced.

Methods

Specimens of *Vallonia* were collected from under stones and dead wood in Kamloops, British Columbia (BC), Canada, and nearby areas in south-central BC. Geographic positions were obtained with a Garmin eTrex Vista HC GPS receiver using the WGS84 geodetic datum. All specimens were placed directly in 95% ethanol and either kept in alcohol for barcoding or dried for storage as dry shells. Specimens of *Vallonia gracilicosta* ($N = 3$, all BC), *Vallonia parvula* ($N = 5$, all Ontario), *Vallonia pulchella* ($N = 2$, both Alberta), and *Vallonia costata* ($N = 19$; Ontario and BC) were deposited at the Biodiversity Institute of Ontario (BIOUG), Guelph, Ontario, Canada. Specimens preserved as dry shells are vouchered in the Invertebrate Collection of the New Brunswick Museum (NBM), Saint John, New Brunswick, Canada.

Anguispira alternata (Say, 1817) (Discidae) ($N = 2$) and *Striatura milium* (Morse, 1859) (Gastrodontidae) ($N = 2$) were selected as outgroups for phylogenetic analysis and also deposited at the Biodiversity Institute of Ontario. All deposited specimens were assigned to the FTMWO or FTMCA project on the Barcode of Life Data Systems (BOLD, http://www.boldsystems.org, Ratnasingham and Hebert 2007). Following protocols in Layton et al. (2018), we used samples of foot tissue for molecular analysis including these steps: (1) amplifying the barcode

region of cytochrome *c* oxidase subunit I (COI) with the universal "Folmer" primers (LCO1490/HCO2198) (Folmer et al. 1994); (2) sequencing; (3) sequence editing and aligning; and (4) sequence quality check. The sequence dataset for the genus *Vallonia* was completed by additional sequence data from GenBank and from various projects on BOLD. The Barcode Index Number (BIN) algorithm was applied to delineate clusters corresponding to operational taxonomic units at the species level (Ratnasingham and Hebert 2013). Specimen collection and sequence information are shown in the Appendix (Table A1).

Molecular phylogenetic analysis was implemented by the Maximum Likelihood method using the Kimura 2-parameter model (Kimura 1980). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach, and then selecting the topology with the superior log-likelihood value. The analysis involved 36 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 344 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura et al. 2013).

Results

New record (barcoded). Canada: BC: Thompson-Nicola Regional District: Kamloops: 1890 McKinley Court, 50.6943° N, 120.3707° W, leg. Forsyth RG, 2015-xi-3; BIOUG27664-G01 and BIOUG27664-F12, 2 spec. (in ethanol), NBM 010448, 21 spec. (dried shells).

Additional new records (not barcoded; identity based on shell morphology). Canada: BC: Thompson-Nicola Regional District. Kamloops: McArthur Island Park, 50.6943° N, 120.3707° W, leg. Forsyth RG, Hutchinson JMC, Reise H, 2013-vii-23; NBM 010449, 5 spec. (dried shells). Kamloops: Peterson Creek ravine complex, below Sa-Hali Secondary School, 50.6628° N, 120.3402° W, leg. Forsyth RG, 2016-xi-7; NBM 010450, 2 spec. (dried shells).

Identification. Among the species of *Vallonia*, the main characters among others used by Pilsbry (1948) for identification included the presence/absence of ribbed sculpture and the number of ribs when present, the development of a thickened lip-rib, the outline of the shell, and whether the suture descends to the adult lip or not. Gerber (1996) provided detailed descriptions of all taxa known to him, which presented the picture that some of these and other characters are much more varied than Pilsbry's (1948) rather simplistic key to species would imply.

We were alerted to some BC material that seemed to have fewer ribs than other material that we have called *V. gracilicosta* and *V. cyclophorella* and somewhat stronger spiral striae on the protoconch. These are characters that Pilsbry (1948) and Gerber (1996) emphasised for *V. costata*. Concerning the shell differences between *V. costata* and *V. gracilicosta*, Gerber (1996: 113) wrote:

> *V. costata* ist weniger eng gerippt, i. d. R. mit weniger kräftigen Kalkrippen, aber mit viel höheren, lamellenförmigen Conchinsäumen; ihre Mündung ist kreisrund, die meist etwas dünnere Lippe tritt nicht über die Mündungsebene vor; ihr letzter Umgang steigt am Ende ab, ohne vorher angestiegen zu sein; der Mundsaum ist weniger stark und plötzlich erweitert; ihr Protoconch zeigt meiste deutliche Spirallinien.
>
> [*Vallonia costata* is less closely ribbed [than *V. gracilicosta*], as a rule with less strong calcareous ribs, but with much higher, lamellar conchiolin edges; its aperture is circular, the usually slightly thinner lip does not occur above the level of the mouth; its last whorl descends at the end, without having risen before; the aperture rim is less strong and expands suddenly; its protoconch shows many clear spiral lines.]

Our Kamloops shells (Fig. 2) conform to specimens of *V. costata* from Ontario and elsewhere that we have seen. That is, these shells are less densely ribbed than the other 2 species and the spiral lines on the protoconch are somewhat more evident.

Barcoding (COI gene sequence) confirmed that these specimens are *V. costata* as they were assigned to the same BIN as all other *V. costata* specimens from Ontario (Fig. 3). Moreover, European specimens of *V. costata* were also found in the same cluster. All nodes at species level in the Maximum Likelihood analysis have support values above 50%, and all morphologically identified species were assigned to corresponding BINs. COI sequence divergence among *Vallonia* species ranged between 2% and 5%.

Discussion

We report the first records of *Vallonia costata* from British Columbia. This is the fifth species of *Vallonia* recorded from the province of BC, although future molecular studies to investigate *V. excentrica/pulchella* and *V. gracilicosta/cyclophorella* (as understood by Forsyth 2004) might show additional cryptic species.

Barcoding allowed us to confirm our initial species identification because *V. costata* from British Columbia was assigned to the same BIN as *V. costata* from Ontario. Genetic divergence among our analyzed *Vallonia* species was generally higher than 2% and the species-level divisions suggested by the BIN on the basis of COI variation also matched the morphological species boundaries. Moreover, specimens from France and Austria clustered together with Canadian *V. costata* specimens in a monophyletic clade, which implies low genetic divergence between the two continents. For all Canadian

Figure 2. *Vallonia costata*, Kamloops, BC (NBM 010448).

Vallonia species, identifications by shell morphology and by barcoding were consistent. At within-species level the 2 individuals of *V. costata* from Kamloops slightly differ in their COI sequences, so they are not clones. Indeed, each individual clusters together with several individuals from Ontario.

If not for the confirmation using COI gene sequence data, we would not feel confident in our initial identification. Due to similarities of shell morphology among species and small size that may hinder study, there may be some uncertainty about identifications in the mostly older literature and collections. It is likely that other species were mistaken for *V. costata* and vice versa in the past. Gerber (1996) developed a key to species in which *V. costata* appears multiple times, which suggests a species with variable shell morphology and/or weak taxonomic characters. However, there is also the possibility, which would require additional study, that *V. costata* is polyphyletic in that it represents an assortment of taxa similar to that what Korte and Armbruster (2003) found for *V. excentrica*.

Gerber (1996: 167, fig. 66) recorded *V. costata* in Canada as far west as Winnipeg, Manitoba, but he admitted that his map likely did not show the true distribution of this species in North America. The range, shown as a hatched area in Gerber's map, appears to extend somewhat further west into at least Saskatchewan. Mozley (1934) reported *V. costata* from several places in Saskatchewan and Alberta, but the whereabouts of Mozley's collections are unknown to us and were not seen by Gerber, so we cannot verify the identifications. As specimens of *V. gracilicosta* in museums may sometimes be misidentified as *V. costata*, it seems possible that Mozley mistook *V. gracilicosta* as *V. costata* as well. Disregarding these old, unconfirmed records, the new BC record is roughly 730 km west of the nearest Canadian record in the Cypress Hills, Alberta (Forsyth and D. A. W. Lepitzki unpublished data), or approximately 1600 km west of the westernmost record known to Gerber (1996), but not the westernmost hatched area in Gerber's (1996: fig. 66) map. In the United States, *V. costata* is recorded as far west as Wyoming (NatureServe 2009), Utah (Gerber 1996), and southern California (Roth and Sadeghian 2006).

The western North American occurrences of *V. costata* are believed to represent introductions (Gerber 1996, Roth and Sadeghian 2003). Gerber (1996) remarked that there is uncertainty whether occurrences around the periphery of its range in both the Nearctic and Palaearctic are autochthonous, but he seems to have been referring to the main areas of distribution, not the scattered records beyond these areas. Based on the scant available evidence, we expect that the new records of *V. costata* in BC represent introductions. *Vallonia pulchella* and/or *V. excentrica* morphospecies were present at all sites; at the McArthur Island site *Cochlicopa lubrica* (O. F. Müller, 1774) and *Zonitoides nitidus* (O. F. Müller, 1774) were present. In Canada, all of these species, including *V. costata*, are often synanthropic.

Acknowledgements

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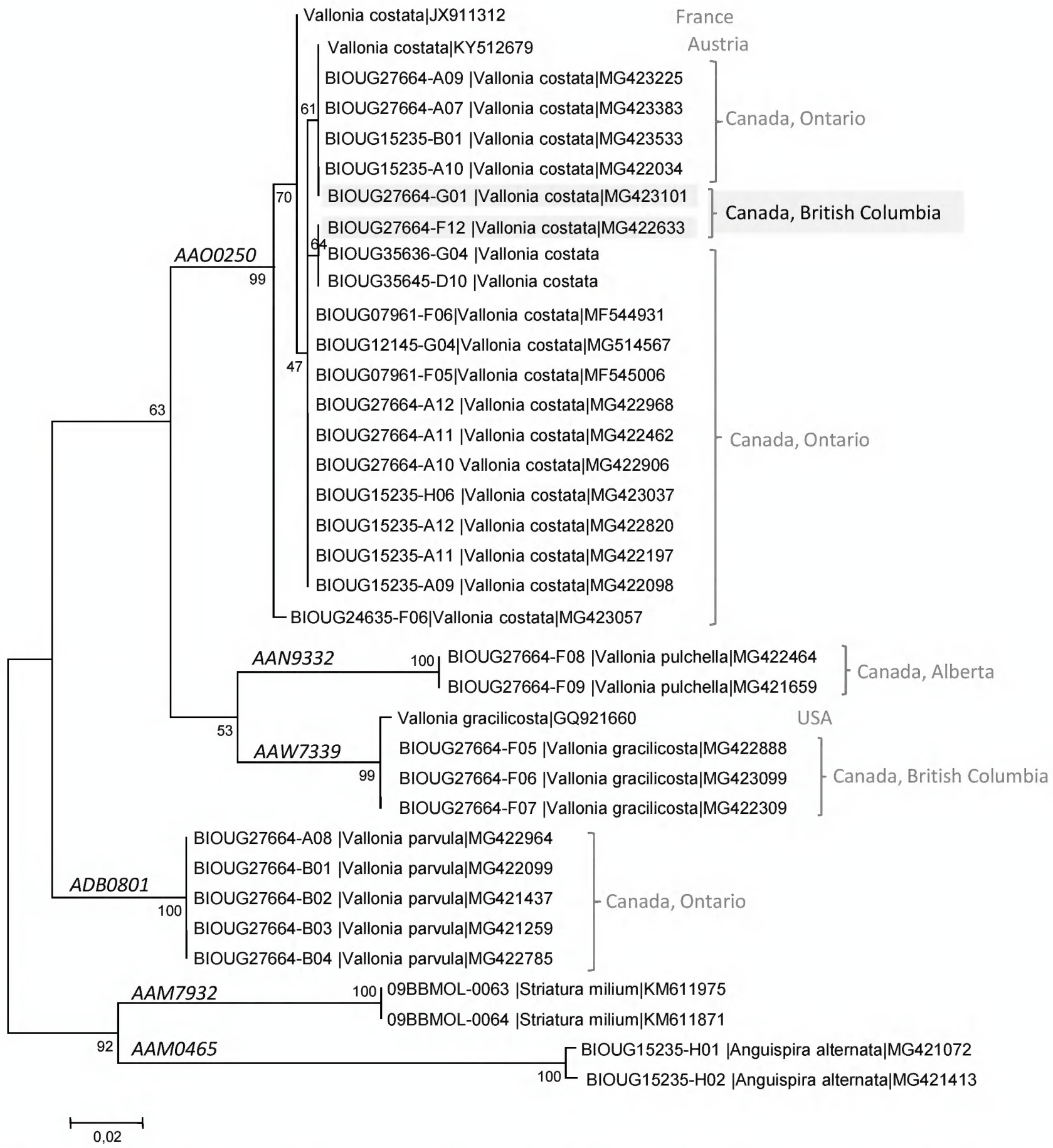

Figure 3. Maximum Likelihood tree of the genus *Vallonia* (Gastropoda, Valloniidae). The tree with the highest log likelihood (–1244.2160) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths proportional to the number of substitutions per site. The Barcode of Life Data Systems sample ID and the Genbank COI gene accession numbers are indicated next to each specimen (left and right, respectively). The Barcode Index Numbers are indicated in italic for each cluster. *Vallonia costata* newly discovered in British Columbia are highlighted in grey.

Görlitz), who helped collect some of the Kamloops specimens reported here, and Jill Crostwhaite and Mhairi McFarlane for access to Nature Conservancy Properties on Pelee Island as well as their support for gastropod sampling. We also thank Parks Canada and Ontario Parks for granting access to protected areas. We thank the reviewers, Timothy Pearce, John Hutchinson, and an anonymous reviewer, as well as the academic editor, for improvements to the manuscript.

Authors' Contributions

RGF and AN shared in writing the text; RGF prepared Figures 1 and 2; AN analysed the COI gene sequences and prepared Figure 3.

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Appendix

Table A1. Specimen and DNA sequence information from Barcode of Life Data Systems (BOLD) and Genbank including Barcoding Identification Number (BIN).

Process ID	Sample ID	BIN in BOLD	COI-5P sequencee length	COI-5P accession	Collection date	Identification	Identifier	Collectors	Locality	Lat.	Long.	Reference
FTMWO197-16	BIOUG27664-F09	AAN9332	549[0n]	MG421659	06-V-2015	*V. pulchella*	R. Forsyth	R. Forsyth	Canada: Alberta: Division No. 15: Municipal Dist. of Bighorn	51.0494	–115.168	
FTMWO196-16	BIOUG27664-F08	AAN9332	647[0n]	MG422464	06-V-2015	*V. pulchella*	R. Forsyth	R. Forsyth	Canada: Alberta: Division No. 15: Municipal Dist. of Bighorn	51.0494	–115.168	
FTMWO201-16	BIOUG27664-G01	AAO0250	658[0n]	MG423101	15-XI-2015	*V. costata*	R. Forsyth	R. Forsyth	Canada: BC: Thompson-Nicola Regional Dist.: Kamloops	50.6537	–120.356	
FTMWO193-16	BIOUG27664-F05	AAW7339	607[0n]	MG422888	17-IV-2015	*V. gracilicosta*	R. Forsyth	R. Forsyth	Canada: BC: Thompson-Nicola Regional Dist.: Savona	50.758	–120.865	
FTMWO200-16	BIOUG27664-F12	AAO0250	476[0n]	MG422633	15-XI-2015	*V. costata*	R. Forsyth	R. Forsyth	Canada: BC: Thompson-Nicola Regional Dist.: Kamloops	50.6537	–120.356	
FTMWO194-16	BIOUG27664-F06	AAW7339	573[0n]	MG423099	17-IV-2015	*V. gracilicosta*	R. Forsyth	R. Forsyth	Canada: BC: Thompson-Nicola Regional Dist.: Savona	50.758	–120.865	
FTMWO195-16	BIOUG27664-F07	AAW7339	571[0n]	MG422309	17-IV-2015	*V. gracilicosta*	R. Forsyth	R. Forsyth	Canada: BC: Thompson-Nicola Regional Dist.: Savona	50.758	–120.865	
SWINP002-16	BIOUG24635-F06	AAO0250	654[0n]	MG423057	28-VII-2015	*V. costata*	A. Telfer	BIObus 2015	Canada: Ontario: Kawartha Lakes: Indian Point Prov. Park	44.6031	–078.829	
FTMWO051-16	BIOUG27664-B04	ADB0801	658[2n]	MG422785	12-VIII-2015	*V. parvula*	R. Forsyth	A. Nicolai, R. Forsyth	Canada: Ontario: Essex Co.: Pelee Island	41.8316	–082.64	
FTMWO050-16	BIOUG27664-B03	ADB0801	658[0n]	MG421259	12-VIII-2015	*V. parvula*	R. Forsyth	A. Nicolai, R. Forsyth	Canada: Ontario: Essex Co.: Pelee Island	41.8316	–082.64	
FTMWO049-16	BIOUG27664-B02	ADB0801	656[0n]	MG421437	12-VIII-2015	*V. parvula*	R. Forsyth	A. Nicolai, R. Forsyth	Canada: Ontario: Essex Co.: Pelee Island	41.8316	–082.64	
FTMWO048-16	BIOUG27664-B01	ADB0801	657[0n]	MG422099	12-VIII-2015	*V. parvula*	R. Forsyth	A. Nicolai, R. Forsyth	Canada: Ontario: Essex Co.: Pelee Island	41.8316	–082.64	
FTMWO047-16	BIOUG27664-A12	AAO0250	454[0n]	MG422968	14-VIII-2015	*V. costata*	R. Forsyth	R. Forsyth	Canada: Ontario: Essex Co.: Pelee Island	41.8037	–082.63	
FTMWO046-16	BIOUG27664-A11	AAO0250	557[0n]	MG422462	14-VIII-2015	*V. costata*	R. Forsyth	R. Forsyth	Canada: Ontario: Essex Co.: Pelee Island	41.8037	–082.63	
FTMWO045-16	BIOUG27664-A10	AAO0250	656[0n]	MG422906	14-VIII-2015	*V. costata*	R. Forsyth	R. Forsyth	Canada: Ontario: Essex Co.: Pelee Island	41.8037	–082.63	
FTMWO044-16	BIOUG27664-A09	AAO0250	656[0n]	MG423225	12-VIII-2015	*V. costata*	R. Forsyth	R. Forsyth, A. Nicolai	Canada: Ontario: Essex Co.: Pelee Island	41.8316	–082.64	
FTMWO043-16	BIOUG27664-A08	ADB0801	658[0n]	MG422964	12-VIII-2015	*V. parvula*	A. Telfer	R. Forsyth, A. Nicolai	Canada: Ontario: Essex Co.: Pelee Island	41.8294	–082.637	
FTMWO042-16	BIOUG27664-A07	AAO0250	658[0n]	MG423383	12-VIII-2015	*V. costata*	R. Forsyth	R. Forsyth, A. Nicolai	Canada: Ontario: Essex Co.: Pelee Island	41.8294	–082.637	
FTMCA429-14	BIOUG15235-B01		652[0n]	MG423533	08-V-2014	*V. costata*	A. Nicolai	A. Nicolai	Canada: Ontario: Wellington Co.: Guelph	43.5332	–080.2269	
FTMCA427-14	BIOUG15235-A11		652[0n]	MG422197	08-V-2014	*V. costata*	A. Nicolai	A. Nicolai	Canada: Ontario: Wellington Co.: Guelph	43.5332	–080.2269	
FTMCA428-14	BIOUG15235-A12		652[0n]	MG422820	08-V-2014	*V. costata*	A. Nicolai	A. Nicolai	Canada: Ontario: Wellington Co.: Guelph	43.5332	–080.2269	
FTMCA506-14	BIOUG15235-H06		652[0n]	MG423037	01-V-2013	*V. costata*	A. Nicolai	M.J. Oldham	Canada: Ontario: Essex Co.: Pelee Island	41.8004	–082.6289	
FTMCA425-14	BIOUG15235-A09		628[0n]	MG422098	08-V-2014	*V. costata*	A. Nicolai	A. Nicolai	Canada: Ontario: Wellington Co.: Guelph	43.5332	–080.2269	
FTMCA426-14	BIOUG15235-A10		652[0n]	MG422034	08-V-2014	*V. costata*	A. Nicolai	A. Nicolai	Canada: Ontario: Wellington Co.: Guelph	43.5332	–080.2269	
RBNII065-13	BIOUG07961-F05	AAO0250	658[0n]	MF545006	14-IX-2013	*V. costata*	A. Nicolai	A.Nicolai	Canada: Ontario: Greater Toronto: Rouge Nat. Urban Park	43.7948	–079.124	
RBNII066-13	BIOUG07961-F06	AAO0250	658[0n]	MF544931	14-IX-2013	*V. costata*	A. Nicolai	A.Nicolai	Canada: Ontario: Greater Toronto: Rouge Nat. Urban Park	43.7948	–079.124	
ROUGE632-17	BIOUG35636-G04	AAO0250	583[0n]		25-VI-2017	*V. costata*	A. Telfer	Rouge NUP BioBlitz Volunteers	Canada: Ontario: Greater Toronto: Markham	43.9371	–079.2285	
ROUGE1457-17	BIOUG35645-D10	AAO0250	583[0n]		25-VI-2017	*V. costata*	A. Telfer	Rouge NUP BioBlitz Volunteers	Canada: Ontario: Greater Toronto: Markham	43.9025	–079.2135	
SSROA3120-14	BIOUG12145-G04		483[0n]	MG514567	09-VI-2013	*V. costata*	A. Telfer	BIObus 2013	Canada: Ontario: Greater Toronto: Rouge Nat. Urban Park	43.808	–079.137	
BBECM001-10	09BBMOL-0063	AAM7932	658[0n]	KM611975	19-VIII-2009	*Striatura milium*	A. Nicolai	BIObus 2009	Canada: New Brunswick: Kent Co.: Kouchibouguac Nat. Park	46.771	–065.006	
BBECM002-10	09BBMOL-0064	AAM7932	658[0n]	KM611871	19-VIII-2009	*Striatura milium*	A. Nicolai	BIObus 2009	Canada: New Brunswick: Kent Co.: Kouchibouguac Nat. Park	46.771	–65.006	
FTMCA501-14	BIOUG15235-H01		652[0n]	MG421072	01-V-2013	*Anguispira alternata*	A. Nicolai	M.J. Oldham	Canada: Ontario: Essex Co.: Point Pelee Nat. Park	41.6825	–082.6828	
FTMCA502-14	BIOUG15235-H02		652[0n]	MG421413	01-V-2013	*Anguispira alternata*	A. Nicolai	M.J. Oldham	Canada: Ontario: Essex Co.: Point Pelee Nat. Park	41.6825	–082.6828	
			655[0n]	KY512679		*V. costata*			Austria: Steiermark: Salzatal: Weichselboden			Harl et al. 2017
GBMLG13323-13			624[0n]	JX911312		*V. costata*			France			Ansart et al. 2014
GBMGP422-10			655[0n]	GQ921660		*V. gracilicosta*			USA			Nekola et al. 2009